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Survival of Zebra Mussels (*Dreissena polymorpha*) and Asian Clams (*Corbicula fluminea*) Under Extreme Hypoxia

by   Milton A. Matthews, Robert F. McMahon,
Center for Biological Macrofouling Research

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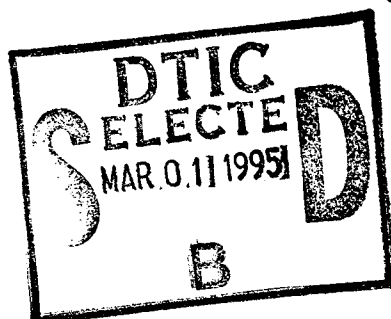
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Survival of Zebra Mussels (*Dreissena polymorpha*) and Asian Clams (*Corbicula fluminea*) Under Extreme Hypoxia

by   Milton A. Matthews, Robert F. McMahon

Center for Biological Macrofouling Research
Department of Biology
University of Texas at Arlington
P.O. Box 19498
Arlington, TX   76019

Final report

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SCALE

500   0   500 m

AREA OF RESERVATION = 2.7 sq km

Contents

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Preface

The Nonindigenous Aquatic Nuisance Prevention and Control Act of 1990 specified that the Assistant Secretary of the Army, Civil Works, will develop a program of research and technology development for the environmentally sound control of zebra mussels (*Dreissena polymorpha*). As a result, the U.S. Army Engineer Waterways Experiment Station (WES) initiated a program to develop control strategies for this species.

This report should be cited as follows:

Matthews, M. A., and McMahon, R. F. (1995). "The survival of zebra mussels (*Dreissena polymorpha*), and Asian clams (*Corbicula fluminea*) under extreme hypoxia," Technical Report EL-95-3, U.S. Army Engineer Waterways Experiment Station, Vicksburg, MS.

1 Introduction

Zebra mussels (*Dreissena polymorpha*) and Asian clams (*Corbicula fluminea*) are both major macrofouling species of municipal potable water, agricultural, industrial, and power station raw water systems in North America (McMahon 1983; Claudi and Mackie 1993). *Corbicula fluminea* is found in 36 of the contiguous states of the United States (Counts 1986) as well as in Hawaii and Mexico (McMahon 1991). Macrofouling by this species has been estimated to cost the power industry over one billion dollars annually (Isom 1986). *Dreissena polymorpha* was introduced to the United States from Europe in 1986 into the Detroit River-Lake St. Clair region of the Great Lakes (Mackie et al. 1989) and has since spread rapidly throughout the Great Lakes, the St. Lawrence River, and the navigable inland waterways of the Mississippi Drainage (Zebra Mussel Information Clearinghouse 1993). A projected cost of two billion dollars has been proposed for the control of *D. polymorpha* over the decade of the 1990s in the Great Lakes alone (Roberts 1990), with this . figure likely to rise exponentially as mussels continue to expand their range in North America.

A further implication of the spread of zebra mussels throughout North American freshwater drainages could be a dramatic increase in the use of molluscicides for mitigation and control of their macrofouling in once-through raw water systems, exacerbating the biocide load already carried by our continental river systems. The likelihood of stricter future regulation of molluscicide usage will require development of readily implemented, cost-effective, environmentally neutral control technologies for freshwater macrofouling bivalves (i.e., *D. polymorpha* and *C. fluminea*).

Both *D. polymorpha* (Mikheev 1964; Mackie et al. 1989) and *C. fluminea* (Fast 1971; McMahon 1983) have been reported to be intolerant of acute hypoxia (i.e., low oxygen concentrations). Neither species can tolerate oxygen-depleted waters below the thermocline of lakes, and both species are excluded from chronically hypoxic waters (for reviews see McMahon 1983 and 1991 and Mackie et al. 1989). Indeed, exposure to oxygen-scavenging chemicals has been reported to be an effective means of mitigating *C. fluminea* macrofouling in raw water intake embayments (Smithson 1986).

Such reports strongly suggest that exposure to anoxia may be an efficient, environmentally neutral means of controlling zebra mussel and Asian clam

fouling in raw water systems. However, a search of the literature revealed no hard information on anoxia tolerance in *C. fluminea* and only a preliminary study on *D. polymorpha* in which the effects of temperature acclimation and accumulating anaerobic toxins were not accounted for (Mikheev 1964). This study was undertaken to more fully detail the anoxia tolerance of both *D. polymorpha* and *C. fluminea* at different experimental and acclimation temperatures. The results are discussed in relation to both species' depth distributions and the efficacy of anoxia as a control strategy.

2 Materials and Methods

Specimens of *D. polymorpha* were collected from Black Rock Lock, on the Niagara River, at its inlet from Lake Erie in Buffalo, NY. Mussels were scraped from a guide wall on the upstream entrance to the lock. Specimens of *C. fluminea* were collected from the water discharge stream of the U.S. Army Corps of Engineers, Lewisville Aquatic Plant Research Facility, which is situated directly below the dam of Lake Lewisville in Denton County, TX. Water from the lake is gravity-fed into a series of experimental ponds that discharge into a canal that empties into the Elm Fork of the Trinity River. Asian clams were collected from the discharge canal, 20 m downstream of the Aquatic Plant Research Facility.

Following collection, zebra mussels were transported overnight, wrapped in moistened paper toweling, in insulated containers with frozen refrigerant packs to keep mussels cool. Mussels arrived in good condition with little observable mortality and were immediately transferred to a 284-ℓ refrigerated holding tank containing aerated, dechlorinated, City of Arlington tap water and maintained at 5 °C without feeding until utilized in experiments. Asian clams were returned to laboratory immersed in water from the collection site within 3 hr of collection. They were placed in a 284-ℓ holding tank in continuously aerated, dechlorinated tap water maintained at a constant temperature of 15 °C on a 12:12-hr light-dark cycle without feeding prior to experimentation. Both species have been successfully held in the laboratory under these conditions for greater than six months without significant tissue biomass loss (Chase and McMahon 1994; Cleland, McMahon, and Elick 1986).

All experiments were initiated within 40 days of collection. Prior to experimentation, samples of individuals from each species were acclimated to 5, 15, or 25 °C for 14 days in plastic tanks holding 17 ℓ of continuously aerated tap water. After acclimation, 30 adult individuals from each species acclimation group were placed in 9-cm-diam by 5-cm-high glass crystallization dishes, which were submersed in approximately 4 ℓ of water held in an air-tight, 5-ℓ plastic container (22 cm long by 22 cm wide by 12 cm high) leaving a 1-ℓ gas head-space. Water in these containers was continuously bubbled with N_2 to deplete media of oxygen. The tolerance of each zebra mussel acclimation group to prolonged anoxia was tested at both 15 and 25 °C ($\pm$ 0.5 °C) in a refrigerated incubator under constant darkness. When Asian clams were similarly tested at 25 °C, results indicated that temperature acclimation did not

affect survival of anoxia (see Chapter 3). Therefore, only one acclimation treatment (15 °C) was tested at the 15 °C test temperature. Control treatments consisting of identical acclimation groups in media bubbled with air rather than N_2 were run concurrently.

Media oxygen saturation in test containers was measured daily with a polarographic silver-platinum oxygen electrode (YSI Model 53). The media was changed in experimental and control containers every two to three days. In all cases, the replacement media was at the test temperature and either aerated (control) or depleted of oxygen by nitrogen bubbling (experimental) before being added to test containers.

The viability of all individuals was tested daily. The posterior mantle edges and siphons of all gaping zebra mussels were gently prodded with the tip of a blunted dissection needle. Individuals which failed to close valves under such stimulation were considered dead. All dead zebra mussels were gaped beyond the limits of normal valve activity. As Asian clams sometimes do not gape on death, viability testing required forcing the tip of a dissection needle several millimeters between the posterior valve margins in the region of the siphons. Living individuals resist needle entry strongly by tight clamping of their valves (McMahon, Shipman, and Long 1992). In contrast, dead clams offer little resistance and fail to close their valves after needle removal. Such testing is not damaging to clams, as indicated by the high survival rate of control individuals. For both species, viability testing required removal of the container from the gas source for not more than 5 min and was carried out on submerged individuals to minimize exposure to oxygen. Viability testing continued until 100-percent mortality was achieved in all samples exposed to anoxia.

3 Results

Throughout the experimental period at test temperatures of 15 and 25 °C, daily measurements indicated that the Po_2 (partial pressure of oxygen) of chamber media bubbled with N_2 never exceeded 5 torr and was usually below 3 torr (i.e., <3 percent of full air O_2 saturation). Thus, test specimens were essentially exposed to anoxic conditions. Zebra mussels in aerated normoxic conditions (i.e., full air saturation with oxygen) readily attached to the walls and floor of holding tanks at 15 and 25 °C regardless of prior temperature acclimation experience. In contrast, zebra mussels held under anoxic conditions never produced byssal attachment threads at either test temperature. In addition, individuals which had attached to the shells of other mussels prior to experimentation routinely released from their byssal holdfasts well in advance of death.

Multiple factor ANOVA showed that temperature acclimation had a significant effect on anoxia tolerance in *D. polymorpha* at both test temperatures (Tables 1 and 2). Mean survival increased with increasing acclimation temperature in *D. polymorpha*, with the mean tolerance of 5 °C-acclimated mussels being significantly lower than that of 25 °C-acclimated mussels (Tukey's Multiple Comparison, P < 0.5, Table 1, Figure 1). Similarly, at 15 °C, mean tolerance time of 5 °C-acclimated mussels was significantly lower than that of either 15 or 25 °C-acclimated specimens. In contrast, there was no difference in the mean anoxia tolerance time of temperature-acclimated groups of *C. fluminea* at a test temperature of 25 °C (Table 3, Figure 1). Therefore, at the subsequent test temperature of 15 °C, anoxia tolerance was only determined for clams acclimated to the median temperature of 15 °C (Table 3, Figure 1).

Specimens of *D. polymorpha* in all three acclimation groups appeared to be considerably less tolerant of anoxia than those of *C. fluminea* (Figures 1 and 2). At a test temperature of 15 °C, mean anoxia tolerance ranged from 229 to 428 hr, in 5 and 25 °C-acclimated mussels, respectively. Mean tolerance of 15 °C-acclimated specimens of *C. fluminea* was 842 hr, twice that of the highest levels recorded in zebra mussels (Figure 1). At a test temperature of 25 °C, mean tolerance times for zebra mussels ranged from 53 to 83 hr over 5 to 25 °C-acclimated individuals. Those for 5 to 25 °C-acclimated specimens of *C. fluminea* at 25 °C ranged from 250 to 283 hr, representing a 3.3- to 4.7-fold longer anoxia tolerance in Asian clams relative to zebra mussels

Table 1
Multiple Factor ANOVA for Testing for Differences in Mean Survival Time for Individuals of *Dreissena polymorpha* Acclimated to 5, 15, and 25 °C and Exposed to Prolonged Anoxia at 25 °C

Source of Variation	Sum of Squares	Degrees of Freedom	Mean Square Error	F-Ratio	Probability
Covariate Shell Length	1,414.82	1	1,414.815	1.306	0.256
Acclimation Temperature	13,637.96	2	6,818.98	6.293	0.0028[1]
Residual	93,185.49	86	1,083.55		
Total	108,773.58	89			

[1] Significant effect at $P \leq 0.05$.

Mean Survival Times for Different Acclimation Groups of *Dreissena polymorpha* Exposed to Prolonged Anoxia at 25 °C with Tukey Multiple Range Analysis for Significant Difference

Acclimation Temp., °C	Mean Hours Survived	n	Standard Error of the Mean	Range	Signif. Diff.[1] (P < 0.05)
5	53.08	30	4.36	9.0 - 104.5	a
15	61.13	30	5.26	9.0 - 129.0	ab
25	82.80	30	7.88	34.0 - 156.0	b

[1] Means with the same letter are not significantly different at the 0.05 level.

(Figure 1). Almost identical patterns of reduced anoxia tolerance in zebra mussels relative to Asian clams were observed when tolerance was recorded as LT_{50} values (i.e., time for 50-percent sample mortality, estimated by Probit Analysis (Bliss 1936)) (Figure 2A) or as SM_{100} values (i.e., time for actual 100-percent mortality of the sample) (Figure 2B).

For specimens of *D. polymorpha*, Multifactor ANOVA revealed a significant correlation between anoxia tolerance and shell length at a test temperature of 15 °C (Table 2), but not at a test temperature of 25 °C (Table 1). Individual least squares linear regression analysis indicated that, at the 15 °C-test temperature, only 5 °C-acclimated individuals displayed a significant size effect with larger individuals having elevated tolerance times (n = 30, r = 0.51, F = 10.2, P = 0.0035). In contrast, correlation between SL and anoxia tolerance in *C. fluminea* was recorded only at a test temperature of 25 °C (Table 3), in which larger clams from the 15 and 25 °C-acclimation groups showed significantly lower survival (15 °C-acclimated: n = 30, r = -0.45, F = 7.14, P = 0.012; 25 °C-acclimated: n = 30, r = -0.37, F = 4.41, P = 0.045).

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Table 2
Multiple Factor ANOVA for Testing for Differences In Mean Survival Time for Individuals of *Dreissena polymorpha* Acclimated to 5, 15, and 25 °C and Exposed to Prolonged Anoxia at 15 °C

Source of Variation	Sum of Squares	Degrees of Freedom	Mean Square Error	F-Ratio	Probability
Covariate Shell Length	150,510.49	1	1,505.49	586.5	0.0175[1]
Acclimation Temperature	576,956.84	2	288,478.42	11.241	<0.0001[1]
Residual	2,207,115.1	86	25,664.13		
Total	2,989,472.0	89			

[1] Significant effect at $P \leq 0.05$.

Mean Survival Times for Different Acclimation Groups of *Dreissena polymorpha* Exposed to Prolonged Anoxia at 15 °C with Tukey Multiple Range Analysis for Significant Difference

Acclimation Temp., °C	Mean Hours Survived	n	Standard Error of the Mean	Range	Signif. Diff.[1] (P < 0.05)
5	228.8	30	24.16	72 - 504	a
15	371.2	30	32.5	96 - 744	b
25	428.00	30	32.71	120 - 768	b

[1] Means with the same letter are not significantly different at the 0.05 level.
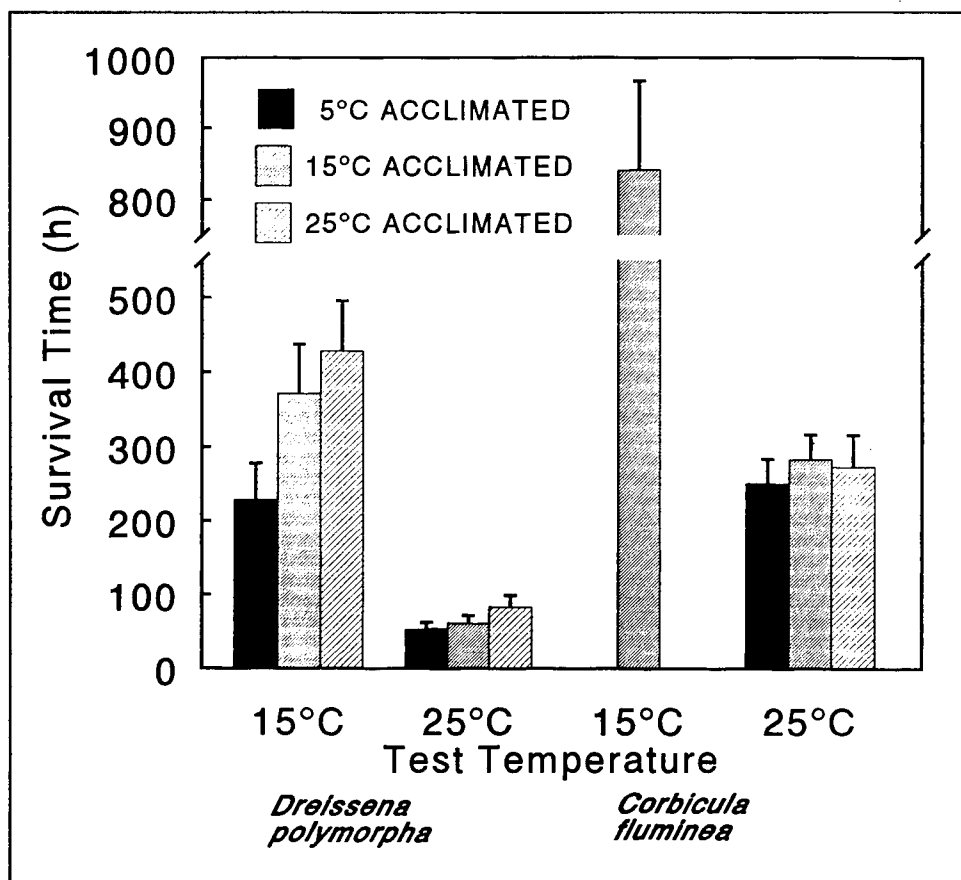
Figure 1. The effects of test temperature and prior temperature acclimation on mean survival time (+ standard error) in hours of zebra mussels (*Dreissena polymorpha*) and Asian clams (*Corbicula fluminea*) under anoxic stress ($Po_2 < 5$ torr) induced by nitrogen bubbling

Table 3
Multiple Factor ANOVA for Testing for Differences in Mean Survival Time for Individuals of *Corbicula fluminea* Acclimated to 5, 15, and 25 °C and Exposed to Prolonged Anoxia at 25 °C

Source of Variation	Sum of Squares	Degrees of Freedom	Mean Square Error	F-Ratio	Probability
Covariate Shell Length	52,557.64	1	52,557.64	5.87	0.0175[1]
Acclimation Temperature	12,667.03	2	6,333.52	0.71	0.50
Residual	761,219.68	85	8,955.53		
Total	830,468.68	88			

[1] Significant effect at $P \leq 0.05$.

Mean Survival Times for Different Acclimation Groups of *Corbicula fluminea* Exposed to Prolonged Anoxia at 25 °C with Tukey Multiple Range Analysis for Significant Difference

Acclimation Temp., °C	Mean Hours Survived	n	Standard Error of the Mean	Range	Signif. Diff.[1] (P < 0.05)
5	250.31	29	16.32	105 - 369	a
15	283.23	30	16.13	105 - 393	a
25	273.017	30	20.65	81 - 405	a

[1] Means with the same letter are not significantly different at the 0.05 level.

Mean Survival Times for Specimens of *Corbicula fluminea* Acclimated to 15 °C and Exposed to Prolonged Anoxia at 15 °C

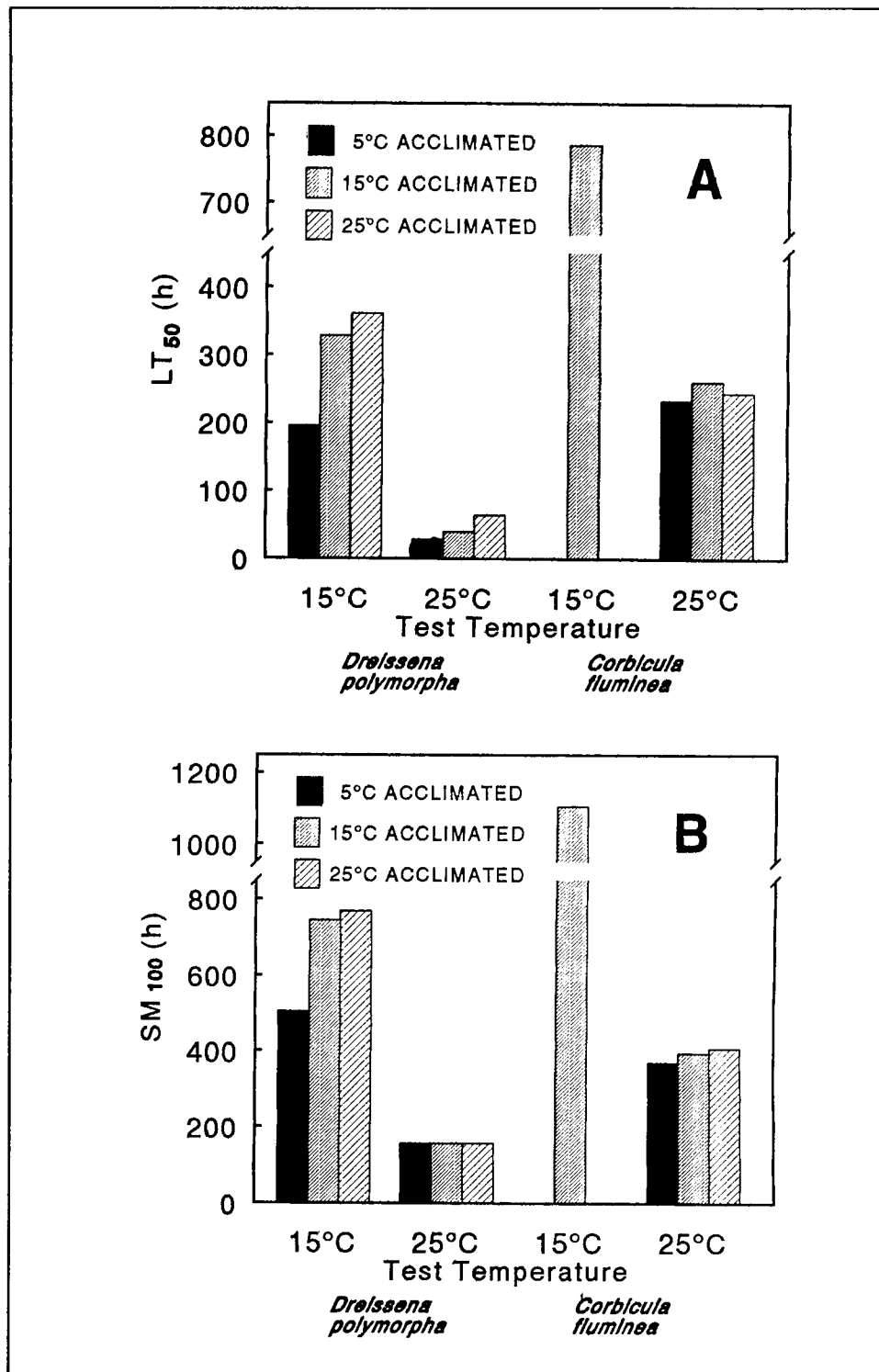| Acclimation Temp., °C	Mean Hours Survived	n	Standard Error of the Mean	Range
15	841.66	29	60.79	240 - 1,248

Figure 2. The effects of test temperature and prior temperature acclimation on survival time in hours expressed as LT_{50} and SM_{100} values for zebra mussels (*Dreissena polymorpha*) and Asian clams (*Corbicula fluminea*) under anoxic stress ($Po_2 < 5$ torr) induced by nitrogen bubbling

4 Discussion

Specimens of *D. polymorpha* were much less tolerant of anoxia than those of *C. fluminea*. Mean tolerance times, LT_{50}, and SM_{100} values for zebra mussels were 2 to 7 times lower than those of Asian clams. Among zebra mussels, those acclimated to 25 °C showed the greatest anoxia tolerance at both test temperatures, surviving a mean of 3.45 days at 25 °C and a mean of 17.8 days at 15 °C. In contrast, Asian clam survival times did not appear to be influenced by temperature acclimation. The highest mean anoxia tolerance times for *C. fluminea* were 11.8 and 35.1 days among 15 °C-acclimated clams at test temperatures of 25 and 15 °C, respectively.

A previous, less extensive study of anoxia tolerance in *D. polymorpha* yielded results similar to those discussed herein. When mussels were enclosed in sealed containers allowing metabolic removal of oxygen, 100-percent mortality was observed among adult specimens within three days at a test temperature of 23 to 24 °C, four days at 20 to 21 °C, and six days at 17 to 18 °C (Mikheev 1964). The somewhat elevated mortality rates reported by Mikheev (1964) may have been due to accumulation of anaerobic metabolic poisons in sealed containers as a consequence of not removing dead specimens. Exposure to relatively low concentrations of sulfide, an anaerobic bacterial decomposition end-product, has been shown to greatly reduce anoxia tolerance in the marine heterodont bivalves, *Macoma secta*, *Macoma nasuta* (Levitt and Arp 1991), *Mulinia lateralis* (Shumway, Scott, and Schick 1983), *Scorbicularia plana*, *Mya arenaria*, *Mytilus edulis*, and *Cardium edule* (Theede et al. 1969).

Mikheev (1964) also reported that smaller individuals of *D. polymorpha* were more susceptible to the lethal effects of prolonged anoxia than were adults, with 100 percent of individuals 1 to 4.9 mm in shell length (SL) succumbing to a 37-hr anoxic exposure at 22 °C while 100 percent of individuals of 20- to 24.9-mm SL survived. In contrast, data from the present study showed no size effects among any acclimation group at a test temperature of 25 °C. At 15 °C, only mussels acclimated to 5 °C showed a significant size effect, with smaller individuals appearing less tolerant of anoxia. As water was changed regularly in these experiments, the size effect reported by Mikheev (1964) may have been due to smaller mussels being more sensitive to accumulation of toxic end-products from anaerobically metabolizing living and decomposing dead mussels in sealed test containers, particularly as the mean

anoxia survival time for smaller specimens in the open, N_2-bubbled media was at least twice that reported by Mikheev (1964).

No data have been previously published regarding the anoxia tolerance of *C. fluminea*. This species shows no capacity to regulate oxygen uptake rate with declining oxygen concentration (McMahon 1979) and has been reported to be unable to tolerate reduced oxygen concentrations associated with discharge of treated sewage (Belanger 1991) and hypolimnetic waters (Fast 1971). The inability of Asian clams to survive for more than 17 days suggests that exposure to anoxia or acute hypoxia during summer months could result in massive population decline.

While data for molluscs are sparse, both zebra mussels and Asian clams appear relatively intolerant of anoxia compared to other freshwater and marine molluscan species. Profundal freshwater sphaeriid clams can survive 4.5 to greater than 200 days of complete anoxia depending on season and temperature (Holopainen 1987), and a freshwater unionid, *Anodonta cygena*, survives at least 7 days of anoxia at room temperature (Zs.-Nagy, Holwerda, and Zander 1982). Specimens of the freshwater pond unionid mussel, *Ligumia subrostrata*, survived anoxia at 22 to 25 °C for greater than 15 days (Dietz 1974). The freshwater, prosobranch, ampullariid snail, *Pomacea lineata*, can survive approximately 40 days of anoxia at 25 °C (Santos, Penteado, and Mendes 1987). In contrast, most freshwater gastropods have anoxia tolerances similar to the range recorded in *D. polymorpha* and *C. fluminea*. For example, the freshwater pulmonate pond snail, *Lymnaea stagnalis*, tolerated anoxia at 20 °C for 2 days, but experienced a latent 100-percent mortality 5 days after return to normoxic conditions (Wijsman, Van der Loot, and Hoogland 1985).

Marine bivalves appear to have a generally similar anoxia tolerance to *D. polymorpha*, but lower tolerance than that of *C. fluminea*. Specimens of *Mulinia lateralis* had LT_{50} values of approximately 11 days at 10 °C, 8 days at 20 °C, and 1.8 days at 30 °C (Shumway, Scott, and Schick 1983), while *Macoma secta* and *M. nasuta* had LT_{50} values of 13 and 18 days, respectively, at 14 to 16 °C (Levitt and Arp 1991). In contrast, the Atlantic oyster, *Crassostrea virginica*, which often encounters hypoxic waters in its estuarine habitats, survived over 28 days of anoxia at 10 °C (Stickle et al. 1989), a tolerance similar to that of the mean of 35 days of anoxia tolerated by *C. fluminea* at 15 °C. Two anoxia tolerant marine bivalves, the mussel, *Mytilus galloprovincialis*, and the ark shell clam, *Scapharca inaequivalvis*, tolerated 15 and 20 days of anoxia, respectively, at 20 °C (de Zwann et al. 1991). The anoxia tolerance of four species of marine bivalves at 10 °C ranged from an LT_{50} of 500-600 hr for the anoxia tolerant *Scorbicularia plana* and *Mya arenaria* to 810 hr for *Mytilus edulis*, to a low of 102 hr for *Cardium edule* (Theede et al. 1969).

Somewhat surprisingly, larger specimens of *C. fluminea* acclimated to 15 and 25 °C were less tolerant of anoxia than smaller individuals at a test temperature of 25 °C. The reason for the reduced anoxia tolerance of larger Asian clams remains unclear; however, older individuals within a *C. fluminea*

population may become senescent with reduced tissue glycogen stores, particularly during and after reproductive periods (Williams and McMahon 1989). Reduction of tissue glycogen levels could make larger, senescent clams less tolerant of anoxia, as glycogen is one of the main sources of anaerobic metabolic substrate in molluscs (de Zwaan 1983). Reduction of anoxia tolerance in larger senescent clams may have occurred at 25 °C because, at this elevated temperature, increased metabolic drive relative to specimens held at 15 °C (McMahon 1979) would lead to more rapid depletion of glycogen energy stores.

A literature search revealed no information on the effects of temperature acclimation on anoxia tolerance in molluscs. Yet, the results of the present study indicated distinct temperature acclimation effects on tolerance in *D. poly-morpha*. At both 15 and 25 °C test temperatures, specimens of *D. polymorpha* acclimated to 5 °C had a lower tolerance of prolonged anoxia than did individuals acclimated to 15 and/or 25 °C (Figures 1 and 2, Tables 1 and 2), while acclimation temperature did not significantly affect mean anoxia tolerance time in specimens of *C. fluminea* (Figures 1 and 2, Table 3). *D. polymorpha* has a typical pattern of metabolic acclimation in which cold-acclimated individuals exhibit higher metabolic rates than do warm-acclimated individuals (Alexander and McMahon 1991). Thus, the reduced anoxia tolerance times of 5 °C-acclimated mussels may have been due to accelerated buildup of toxic anaerobic end-products to lethal levels as a result of their elevated metabolic rates. In contrast, temperature acclimation did not affect anoxia tolerance in *C. fluminea*. Like *D. polymorpha*, *C. fluminea* has a typical pattern of metabolic temperature acclimation (McMahon 1979). However, the greatly extended anoxia tolerance times of this species (11.8 days at 25 °C and 35.1 days at 15 °C) may have allowed previously acclimated specimens to fully reacclimate to test temperatures, thus masking any effect of prior temperature acclimation on anoxia tolerance.

Zebra mussels in media bubbled with air rapidly made byssal holdfasts while those under anoxia never produced byssal threads. Furthermore, the majority of previously attached zebra mussels were observed to drop their byssal holdfasts well in advance of death. Similar inhibition of byssal holdfast production under anoxic conditions has been reported in the marine mussel, *Mytilus edulis* (Ravera 1952).

5 Conclusions

Exposure to prolonged anoxia has been utilized to mitigate Asian clam fouling in the raw water intake embayments of a power plant. When the embayment was off-line, oxygen was scavenged from water above the clams by pumping sodium-meta-bisulfite ($Na_2S_2O_5$) and hydrogen sulfide (H_2S) to the embayment floor and leaving it undisturbed for 60 to 72 hr at water temperatures above 21 °C (Smithson 1986). The rapid kill achieved by this method compared to death by anoxia alone documented in this paper was likely due to concomitant exposure to sulfide, which is known to reduce anoxia tolerance in other species of molluscs (Theede et al. 1969; Shumway et al. 1983; Levitt and Arp 1991). Similar embayment treatments may result in more rapid mitigation of *D. polymorpha* macrofouling, as this species is much less anoxia-tolerant than *C. fluminea*. However, because *D. polymorpha* fouling occurs on all hard surfaces within an embayment, and not just on the embayment floor as occurs with *C. fluminea* fouling (Smithson 1986), application procedures will require adequate distribution of oxygen-scavenging chemicals throughout the embayment water column.

Exposure to anoxia has been previously suggested as an environmentally acceptable, nonchemical control methodology for *D. polymorpha* macrofouling (McMahon 1990; Claudi and Mackie 1993; Electric Power Research Institute 1993). Oxygen depletion can be achieved within a raw water system by injecting sodium-meta-bisulfite and/or hydrogen sulfide immediately prior to valving it off. Alternatively, the system may be valved shut and the natural oxygen demand of mussels and other fouling organisms utilized to create anoxic conditions in static, stagnant water (Mikheev 1964). At higher temperatures (>20 °C), 100-percent mortality could be achieved within 12 days of anoxia for *D. polymorpha* and within 25 days for *C. fluminea*. It is highly likely that utilization of hydrogen sulfide as an oxygen scavenger (Levitt and Arp 1991; Shumway, Scott, and Schick 1983) or the accumulation of naturally produced toxic anaerobic end-products and sulfide from dying mussels in an off-line static system (Mikheev 1964) would significantly reduce the anoxic exposure required for 100-percent mitigation, particularly at higher temperatures (>20 °C). In contrast, the greatly extended anoxia tolerance of both *D. polymorpha* and *C. fluminea* at temperatures below 15 °C is likely to render anoxic treatment unsuitable for bivalve macrofouling control under low temperature conditions at most facilities. Indeed, at 5 °C, specimens of *D. polymorpha* survived up to 62 days anoxia while those of *C. fluminea*

showed no mortality over a 12-week period (Matthews and McMahon unpublished results).

Use of anoxia for mitigation of bivalve fouling will lower pH in treated raw water systems due to release of organic acids and hydrogen sulfide from decomposing mussels and other fouling organisms. Injection of sodium-metabisulfite or hydrogen sulfide as oxygen scavengers will further reduce pH, increasing the rate of corrosion in metallic piping (Claudi and Mackie 1993). Further, static lay-up of systems may stimulate growth of sulfate- reducing bacteria (*Desulfovibrio* and *Desulfomaculum*), which reduce sulfate (SO_4^{2-}) to sulfide (S^{2-}) ions that can attack cast iron, carbon, and low-alloy steels (Licina 1988; Claudi and Mackie 1993). Thus, application of anoxia as a mitigation treatment should not greatly exceed durations required to kill fouling bivalves. Indeed, where fouling infestations of Asian clams or zebra mussels have been allowed to achieve high densities, the potential for massive production of sulfide by decomposing bivalve bodies may preclude use of anoxia as an initial mitigation treatment. Periodic annual or biannual anoxia treatment should prevent excessive accumulation of a large fouling biomass, making mitigation of *D. polymorpha* or *C. fluminea* macrofouling both highly cost-effective and environmentally acceptable.

It has been reported that populations of *D. polymorpha* (Mackie et al. 1989; McMahon 1990) and *C. fluminea* (McMahon 1983) do not normally extend below the thermocline (i.e., zone of thermal discontinuity) into the hypolimnetic waters of lentic (i.e., lake) habitats. Due to thermal stratification, hypolimnetic waters are often highly hypoxic during summer months. The results of this research suggest that the relatively poor tolerance of *D. polymorpha* and *C. fluminea* to prolonged anoxia acts to restrict both species to shallow, well-oxygenated, surface waters. Fast (1971) showed that artificial aeration allowed *C. fluminea* to invade the deeper hypolimnetic waters of a small lake from where it had been previously excluded. As adults, juveniles and larval stages of *D. polymorpha* (Mackie et al. 1989; McMahon 1990; Claudi and Mackie 1993) and *C. fluminea* (McMahon 1983) are excluded from deeper, hypoxic, hypolimnetic waters. Thus, placement of intake structures below the thermocline of cooling water reservoirs has been utilized as an effective means of controlling raw water system fouling by these species (McMahon 1990; Claudi and Mackie 1993). Modification of existing intake structures to allow periodic drawing of anoxic water from below a source water's thermocline during summer months could serve to mitigate zebra mussel or Asian clam infestations. Periodic application of anoxic, subsurface water could minimize the microbially induced corrosion (M.I.C.) associated with anoxia produced by static lay-up (Licina 1988) by flushing anaerobically produced sulfides and organic acids into the discharge. Indeed, application of anoxic, subsurface water might cause fouling mussels to release from byssal holdfasts and be flushed from the system well in advance of death, as was observed in the present study.

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REPORT DOCUMENTATION PAGE

Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden, to Washington Headquarters Services, Directorate for Information Operations and Reports, 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302, and to the Office of Management and Budget, Paperwork Reduction Project (0704-0188), Washington, DC 20503.

1. AGENCY USE ONLY (Leave blank)	2. REPORT DATE January 1995	3. REPORT TYPE AND DATES COVERED Final report

4. TITLE AND SUBTITLE
Survival of Zebra Mussels (*Dreissena polymorpha*) and
Asian Clams (*Corbicula fluminea*) Under Extreme Hypoxia

5. FUNDING NUMBERS

6. AUTHOR(S)
Milton A. Matthews, Robert F. McMahon

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
Center for Biological Macrofouling Research
Department of Biology, P.O. Box 19498
University of Texas at Arlington
Arlington, TX 76019

8. PERFORMING ORGANIZATION REPORT NUMBER
Technical Report
EL-95-3

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Corps of Engineers
Washington, DC 20314-1000;
U.S. Army Engineer Waterways Experiment Station
3909 Halls Ferry Road, Vicksburg, MS 39180-6199

10. SPONSORING/MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES
Available from National Technical Information Service, 5285 Port Royal Road, Springfield, VA 22161.

12a. DISTRIBUTION/AVAILABILITY STATEMENT

Approved for public release; distribution is unlimited.

12b. DISTRIBUTION CODE

13. ABSTRACT *(Maximum 200 words)*

Zebra mussels (*Dreissena polymorpha*) and Asian clams (*Corbicula fluminea*) were exposed to acute hypoxia (P_{O_2} < 5 torr or 3 percent of full air oxygen saturation) in order to determine anoxia tolerance and assess O_2 as an efficacious, nonchemical, environmentally neutral, macrofouling control strategy. Zebra mussels were collected from Black Rock Navigation Lock on the Niagara River in Buffalo, NY, and Asian clams from the U.S. Army Corps of Engineers, Aquatic Ecosystems Research Facility, Lewisville Lake, Denton County, TX. Adults of both species were acclimated to 5, 15, or 25 °C for 14 days prior to anoxia tolerance testing. After acclimation, paired samples of 30 individuals from each acclimation group were placed in separate 5-ℓ air tight plastic containers (22 cm by 22 cm by 12 cm) with 4 ℓ of dechlorinated tap water, leaving a gas head-space of 1 ℓ. Paired samples of both species were held in either aerated (control) or anaerobic water (treatment), which was made anoxic by continuous gassing with N_2, and held at test temperatures of either 15 or 25 °C. Medium O_2 concentrations and mortality were recorded daily. Dead individuals were removed and their shell lengths (SL) measured to the nearest 0.1 mm. Container medium was replaced every three days with previously deoxygenated or oxygenated water at the test temperature as appropriate. Negligible mortality (<3 percent) was recorded in all aerated control samples of both species.
(Continued)

14. SUBJECT TERMS
Corbicula   Zebra mussels
Hypoxia
Nonindigenous species

15. NUMBER OF PAGES
25

16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
UNCLASSIFIED	UNCLASSIFIED		

13. (Concluded).

Mean anoxia tolerance (MAT) for *D. polymorpha* was significantly (P < 0.05) affected by acclimation temperature at both 15 and 25 °C-test temperatures. At 25 °C, MAT was 53.1 hr (S.D. = ±23.9), 61.1 hr (±28.8) and 82.8 hr (±43.1) for 5-, 15-, and 25 °C-acclimated mussels, respectively. At the 15 °C test temperature, the corresponding values were 228.8 hr (±132.3), 371.2 hr (±178.0) and 428.0 hr (±179.2) for 5-, 15- and 25 °C-acclimated specimens, respectively. Higher anaerobic metabolic rates among 5 °C-acclimated mussels may have induced more rapid accumulation of toxic anaerobic end-products, leading to reduced survival. Temperature acclimation did not affect MAT in *C. fluminea*. At a test temperature of 25 °C, MAT ranged from 250.3 hr (±87.9) to 283.2 hr (±88.4) across the three acclimation groups, and at a test temperature of 15 °C, this value was 841.7 hr (±327.4) in 15 °C-acclimated clams (only 15 °C-acclimated specimens were tested). Within the size range tested (*D. polymorpha* = 12 to 34 mm, *C. fluminea* = 10 to 30 mm) there was little evidence of a consistent size effect on anoxia tolerance other than a trend toward decreasing tolerance among larger individuals of *C. fluminea* at a test temperature of 25 °C. *Dreissena polymorpha* and *C. fluminea* appear to be less tolerant of anoxia than the majority of native North American freshwater bivalves, suggesting that exposure to anoxic conditions may be an efficacious nonchemical control technology for either species, and particularly for *D. polymorpha* as it proved 2 to 7 times less tolerant of anoxia than did *C. fluminea* depending on test and acclimation temperatures. Low tolerance of anoxia may represent a physiological limitation restricting the distribution of both species to well oxygenated rivers and the epilimnetic zone of lakes.